



For research use only

**Protocol** 

# CD3/CD28 Streptamer® Kit

Cat. no. 6-8920-050

mouse, for T cell expansion

# 1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

## Kit components:

Cat. no.	Product	Quantity
6-8913-125	CD3 Fab-Strep, mouse, lyophilized, 125 µg	1
6-8914-125	CD28 Fab-Strep, mouse, lyophilized, 125 µg	1
6-8912-050	Strep-Tactin® Multimer, 500 µl	1
6-6325-001	Biotin stock solution, 100 mM, 1 ml	1

**Specifications:** For stimulation and expansion of **5** x **10**<sup>7</sup> murine T cells

Required: Appropriate cell culture medium (recommended: RPMI + 10% FCS + 1% Pen-Strep +

50U/ml IL-2); buffer (e.g. PBS)

Storage: Store all components at 2 - 8 °C. Store reconstituted Fab-Streps at -80 °C.

**Stability:** 6 months after shipping.

Shipping: Blue ice

Warnings: Products are not classified as hazardous according to (EC) No 1272/2008 [CLP].

Material Safety Data Sheets are provided.

## 2. INITIAL PREPARATIONS

#### 2.1. Reagent preparation

Volumes are suitable for stimulation and expansion of **5 x 10**<sup>5</sup> T cells. For different cell numbers, adapt volumes according to **Table 1**.

- 2.1.1. Dissolve each Fab-Strep in 500 µl buffer. Store Fab-Strep suspensions in aliquots at -80 °C.
- 2.1.2. Combine 5 μI CD3 Fab-Strep with 5 μI CD28 Fab-Strep and 5 μI of Strep-Tactin® Multimer in a 0.5 mI tube. Incubate the mixture for at least 20 min at 4 °C under constant agitation (e.g. using a roller mixer) to generate CD3/CD28 Streptamer® complexes.



We recommend preparing CD3/CD28 Streptamer® complexes freshly for each experiment. If necessary, store pre-mixed components at 4 °C. **Do not freeze!** 

#### 2.2. Cell preparation

**2.2.1.** Isolate primary T cells or subsets of interest from murine splenocytes and resuspend in a buffer suitable for cells.



Alternatively, splenocytes can be used (optionally with red blood cell lysis prior to step 2.2.2.).

- **2.2.2.** Determine the number of T cells (either in pre-isolated population or within whole splenocyte population)
- 2.2.3. Resuspend cells in cell culture medium at a concentration of **5** x **10**<sup>5</sup> <u>T cells</u> per 1 ml (optimal conditions should be titrated).

Table 1: Recommended volumes for different T cell numbers

	96-well	48-well	24-well
T cell number	5 - 8 x 10 <sup>4</sup>	2 - 5 x 10 <sup>5</sup>	0.5 - 1 x 10 <sup>6</sup>
Volume culture medium [ml]	0.1 - 0.2	0.5 - 1.0	1.0 - 2.0
CD3/CD28 Streptamer® premix [µI]	3	15	30
Biotin [µl]	1 - 2	5 - 10	10 - 20



Biotin is required if you want to terminate the stimulation at a certain point during the expansion period or remove CD3/CD28 Streptamers from the cells. See **3.3.** for details.

## 3. PROTOCOL

#### 3.1. T cell activation

- 3.1.1. Seed  $2 5 \times 10^5$  purified T cells in 0.5 1 ml cell culture medium in a 48-well plate.
- 3.1.2. Add 15 μl CD3/CD28 Streptamer® premix to the cells and mix gently. Incubate cell suspension in a humidified CO₂ incubator at 37 °C, according to your experimental setup.
- **3.1.3.** Harvest activated T cells and use directly for further analysis.



Activation markers CD25 and CD69 should be upregulated after 48 h.

### 3.2. T cell expansion

- **3.2.1.** Examine culture daily regarding cell size, shape and cluster formation (using a microscope). Count the cells (at least every two or three days) to evaluate cell density (should not exceed 1 x 10<sup>6</sup> cells/ml). If cell medium turns yellow or cell density is too high, split cultures back to a density of 0.5 1 x 10<sup>6</sup> cells/ml into a new plate of appropriate size.
- 3.2.2. Restimulation of the cells might be necessary after a couple of days in culture (signs of exhaustion typically after 10 14 days). Repeat therefore from step 3.1.2.

#### 3.3. Termination of stimulation/removal of CD3/CD28 Streptamers

- **3.3.1.** Add biotin directly to the culture medium containing the activated T cells (volumes see Table 1). The final concentration of biotin should be 1 mM.
- **3.3.2.** Incubate cell suspension for **30 min at room temperature**.
- **3.3.3.** Harvest cells by resuspension and transfer them to a 15 ml reaction tube. Add **10 ml** of culture medium for dissociation of CD3/CD28 Streptamers. Collect cells by centrifugation at **300 x g** for **6 10 min**.
- **3.3.4. Discard supernatant completely** and repeat washing with **10 ml** cell culture medium. Cells are now ready for further downstream analyses



For T cell expansion, cells should be stimulated at least for 24 h.



Check our Downloads page

https://www.iba-lifesciences.com/resources/download-area/

for the latest version of this protocol



Info on warranty / licensing and trademarks available at:

www.iba-lifesciences.com/patents-licenses-trademarks/



If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!